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Effect of Incubation Temperature on the Somatic Development of the Snail-Eating Turtle *Malayemys macrocephala*

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Abstract The rising average global temperature can lead to changes both in the physical and biological environments and affect the survival of organisms. Freshwater turtles are considered to be susceptible species since their development is dependent on incubation temperature. In Thailand, although several species of freshwater turtle are present, the extent of their susceptibility to temperature change is unknown due to the lack of information on their developmental patterns. This research, therefore, is aimed at examining the effects of temperature on somatic development in *Malayemys macrocephala*, a native species and the most common freshwater turtle in Thailand. Turtle eggs were collected from rice fields in the central part of Thailand during December 2011 to February 2012 inclusive. Eggs (237–238 per condition) were incubated in microprocessor-controlled incubators at three different temperatures (26 °C, 29 °C and 32 °C) with a relative humidity in excess of 80%. Each week, until the remaining eggs hatched, eggs were randomly selected, removed and dissected to reveal the developing embryo to screen for developmental stage and any abnormalities. The incubation period (lay to hatch) was not significantly different among the three temperatures (115 ± 11.3 d, 115 ± 20.3 d and 109 ± 17.8 d, respectively), but the growth patterns, as indicated by the stages of development and carapace lengths, were significantly different. At a high temperature (32 °C), turtle embryos showed a significantly faster growth rate than at the intermediate and low temperatures (29 °C and 26 °C), but had a significantly (over 3.3-fold) higher incidence of developmental abnormalities (especially deformed bodies) than at the lower temperatures. Overall, the results indicate that incubation temperature is an important variable affecting the somatic development of this tropical freshwater turtle species, whilst abnormalities in the embryonic body may be a sensitive indicator of extreme thermal stress.

Keywords abnormality, egg incubation, embryonic stage, freshwater turtle, growth

1. Introduction

The Intergovernmental Panel on Climate Change (IPCC, 2007) reported that global warming, a gradual increasing in the average temperature on the earth, can lead to changes in both the physical and biological environment and affect the survival of organisms. Organisms that cannot adjust themselves to suit the modified environment (or move to another cooler region if available) may decline in number, range and become extinct (Walther *et al*., 2002). In reptiles, temperature is an important ecological factor (Zug *et al*., 2001), and the incubation temperature has been demonstrated to have profound effects on embryonic development (Deeming and Ferguson, 1991). Incubation at a high temperature can accelerate embryonic development compared to that at a lower temperature (Pina and Larriera, 2002; Georges *et al*., 2005).

In freshwater turtles, incubating eggs at different temperatures can affect the incubation period and the pattern of development (Pieau and Dorizzi, 1981; Packard *et al*., 1987). An increased incubation temperature resulted in the earlier hatching of many reported species (Ewert, 1979, 1985), and is also an important variable affecting the hatching rate and development of freshwater turtles (Deeming and Ferguson, 1991; Grant *et al*., 2003),

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and can determine the sex ratio of a turtle population (Bull *et al.*, 1990; Willingham, 2005). An ecological model predicted that climate change may exhibit a long-term impact on freshwater turtle populations (Parrott and Logan, 2010).

In addition, the population dynamics of the freshwater turtle may be affected by developmental abnormalities (Davy and Murphy, 2009). Although developmental abnormalities of varying degrees of severity are not uncommon in wild populations of freshwater turtles (Ewert, 1979; MacCulloch, 1981; Pavaliko, 1986; Bell *et al.*, 2006), the thermal conditions during incubation have been shown to have significant effects on the embryonic growth and development in turtles (Deeming and Ferguson, 1991). Incubation at temperatures below or above the optimal thermal range for significant periods of time can result in an increased frequency and or severity of embryonic malformation (Booth, 2006).

Although several species of freshwater turtles are present in Thailand (Nutphand, 1979; Thirakhupt and van Dijk, 1994), the extent of their susceptibility to temperature change is unknown due to a lack of information on their development patterns. The snail-eating turtle, *Malayemys macrocephala*, is one of the native species and is currently the most common freshwater turtle species in Thailand (Brophy, 2004). Sufficient baseline information about this species is available in regard to its natural history (Nutphand, 1979), population biology (Srinarumol, 1995), and ecotoxicology (Keithmaleesatti, 2008). This research, therefore, is aimed to examine the effects of the incubation temperature on the somatic development of *M. macrocephala* in order to provide a prediction of the potential effects of an increased regional temperature on this native freshwater turtle species.

2. Material and Methods

2.1 Turtle egg collection Visual encounter surveys for nests of *M. macrocephala* were carried out from December 2011 to February 2012 (inclusive) in rice fields (UTM Zone 47P: 0653086-0659077 and 1583552-1591014; Figure 1) at Bang Ban district, Phra Nakhon Si Ayutthaya province. Located in the central part of Thailand, this is an important breeding ground for this turtle species and so adverse effects on this population would likely represent most of the Thai population (Keithmaleesatti, 2008). Based on five field surveys, a total of 712 eggs from 126 clutches were collected from paddy ridges and the edges of canals and pools. During the surveys, the average ambient temperature was 30.72 °C ± 4.65 °C (ranging from 20.30 °C to 41.80 °C), while the average nest temperature was lower at 28.47 °C ± 3.80 °C (ranging from 20.50 °C to 36.50 °C). However, this still indicated a potential exposure to wide range of developmental temperatures. Collected eggs

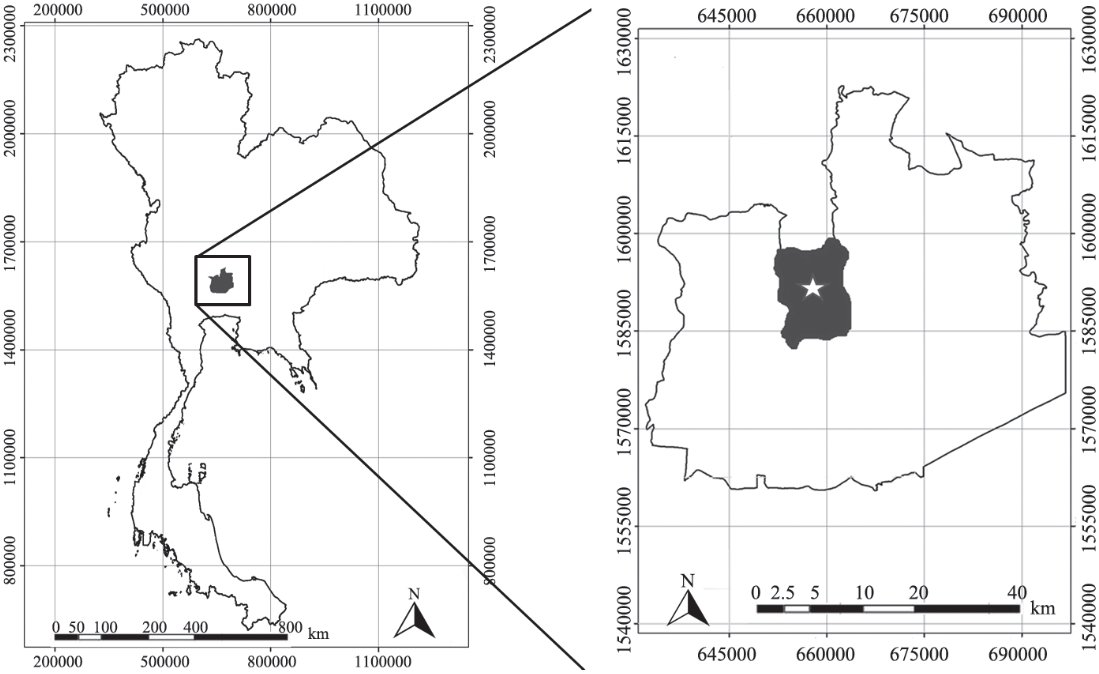

Figure 1 Egg collection area (star) at Bang Ban district, Phra Nakhon Si Ayutthaya province, Thailand (UTM Zone 47P: 0653086-0659077 and 1583552-1591014).

were individually numbered, placed in a Styrofoam box and transported to the laboratory at the Department of Biology, Faculty of Science, Chulalongkorn University.

2.2 Turtle egg incubation In the laboratory, eggs were cleaned, weighed and randomly selected into three groups. In each group of eggs, the eggs were placed in plastic boxes containing moistened vermiculite (1:1 (w/v) vermiculite: distilled water). Since the majority of turtle nests (71 out of 126 nests) showed a nest soil temperature in the range of 26–32 °C, an incubation temperature range of 26–32 °C (as 26 °C, 29 °C and 32 °C for the three groups, respectively) was used in this study. Each group of eggs were kept in a microprocessor-controlled incubator (Siam Incubators System, Bangkok, Thailand) at the selected temperature of 26 °C (237 eggs), 29 °C (237 eggs) and 32 °C (238 eggs). A tray of water was placed inside each incubator to control relative humidity in excess of 80%. The temperature and relative humidity inside the box were monitored using a data logger (HAXO-8, LogTag Recorders, Auckland, New Zealand). Eggs were randomly sampled, weighed and dissected on a weekly basis until they hatched. The number of days required for a turtle embryo to hatch was defined as the incubation period.

2.3 Growth and development of turtle embryos After dissection, the developmental stage of each turtle embryo was studied in reference to the widely used developmental stages of *Chelydra serpentina* (Yntema, 1968) and *Pelodiscus sinensis* (Tokita and Kuratani, 2001). Body length was measured by a vernier caliper (Mitutoyo, accuracy 0.01 mm) and used as an indicator of the size of the embryo. The crown-rump length (CRL) was measured at developmental stages 3 to 14 and the carapace length (CL) was measured at developmental stages 15 to 26 (Figure 2). After measurement, embryos were subjected to euthanasia by injection with sodium pentobarbital (600 mg/kg) intraperitoneally, fixed in Davidson's fixative for 24 h and preserved in 70% (v/v) ethanol.

2.4 Developmental abnormalities of turtle embryos Since developmental abnormalities can occur with turtle embryos, the fixed samples were examined for their visual external morphology and any discernible abnormalities were classified into the three widely used categories of deformity types in embryos of *Chelydra serpentina* and *Chrysemys picta* (Bell *et al.*, 2006) as follows. Type 1 deformities are minor and are not likely to affect survival, including a narrow body (Figure 3 A). Type 2 deformities are moderate and can lower the chance of survival, such as 1) scoliosis, 2) missing eye, 3) flat skull and misshaped carapace, and 4) a deformed body (Figure 3 B–E), whilst type 3 are deformities that greatly reduce the chance of survival and so are essentially lethal ones, including dwarfs (Figure 3 F).

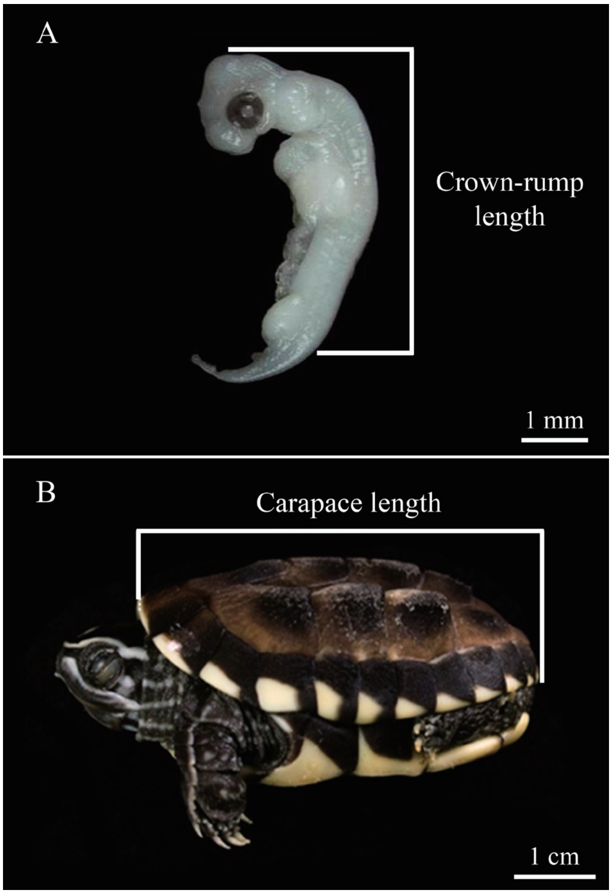

Figure 2 Measurement for body length as an indicative of size of the (A) crown-rump length (CRL) for embryonic stages 3 to 14, and (B) the carapace length (CL) for embryonic stages 15 to 26.

2.5 Statistical analyses All data were tested for compliance with a normal distribution and homogeneity of variance prior to parametric statistical analysis. The mean incubation periods were compared among the different incubating temperatures using one-way analysis of variance (ANOVA). Relationships between the incubation time *vs.* embryonic stage and the incubation time *vs.* CL were analyzed by logistic regression analysis. Afterwards, the slopes of the trend lines were compared among temperatures by ANOVA followed by Tukey's multiple comparisons.

Eggs/embryos from each incubating temperature were classified into the three categories of 1) non-fertile eggs, 2) normal embryos, and 3) deformed embryos. A 3 × 3 contingency table was used to determine the association between temperature (26 °C, 29 °C and

32 °C) and normality in development (non-fertile egg, normal embryo and deformed embryo).

In all cases statistical significance was accepted at a $P < 0.05$ level, and all statistical procedures undertaken in this study was performed according to Zar (1998).

3. Results

3.1 Effect of temperature on the incubation period

Table 1 shows that the incubation period for *M. macrocephala* eggs was fairly broad, ranging from 78 to

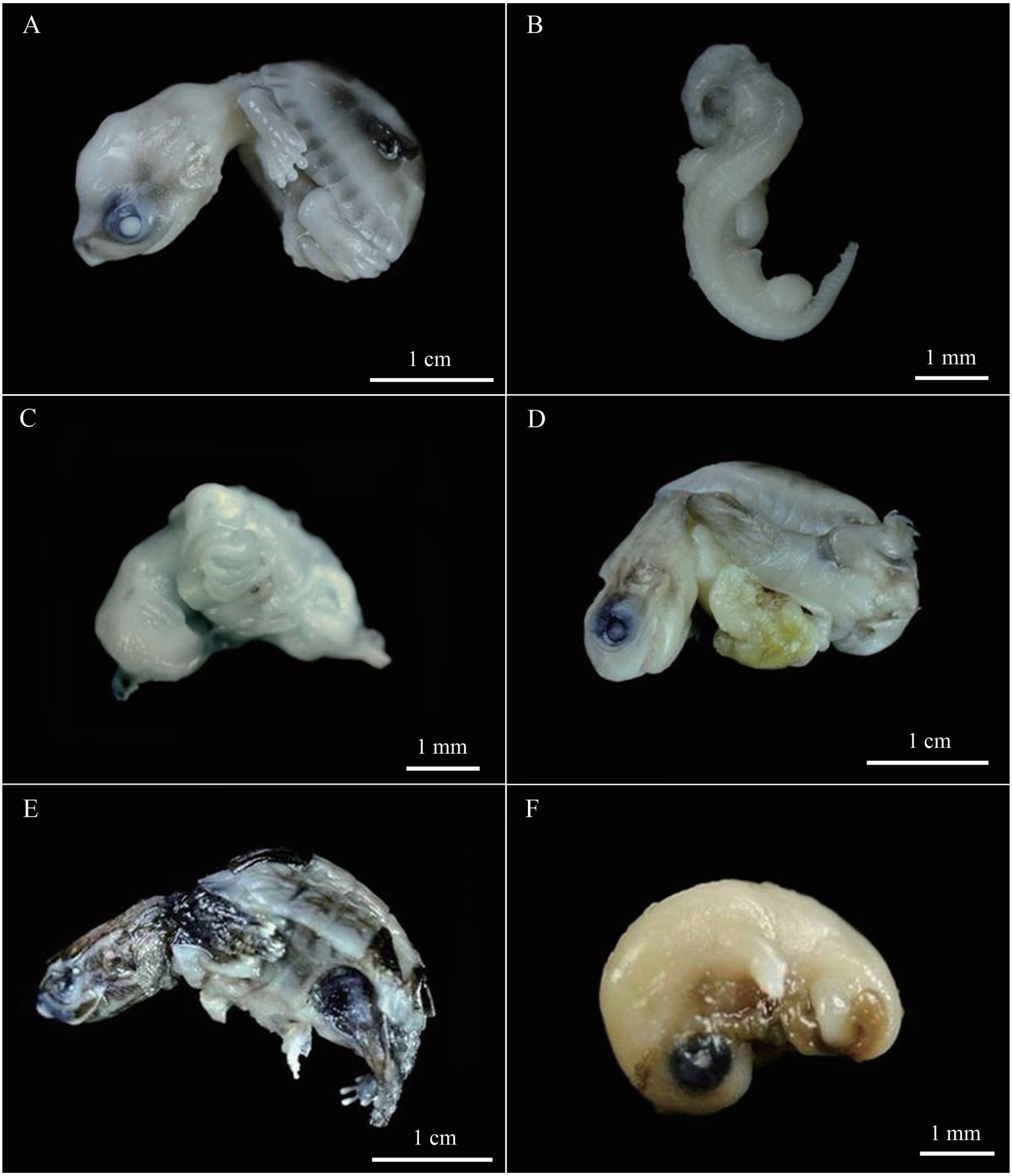

Figure 3 Types of deformities found in *M. macrocephala* embryos: Type 1: (A) narrow body. Type 2: (B) scoliosis, (C) missing eye, (D) flat skull and misshaped carapace, and (E) deformed body. Type 3: (F) dwarf.

150 d. However, although the average hatching time was numerically slightly shorter when incubated at 32 °C than at 26 °C or 29 °C, there was no statistically significant difference in hatching times among the three temperatures (one-way ANOVA, $P > 0.05$), with the mean incubation period ranging from 109 to 115 d.

3.2 Effect of temperature on the growth and development of turtle embryos Development of *M. macrocephala* embryos was monitored from the 2nd week to the 24th week of incubation (Table 2). Significant logistic regressions between the incubation time and the developmental stage of *M. macrocephala* were found for all three incubating temperatures (26 °C, 29 °C and 32°C) with R^2 values of 0.95, 0.89 and 0.85, respectively (Figure 4).

The growth of *M. macrocephala* embryos was assessed from the CRLs for embryonic stages 3 to 14 and the CLs for embryonic stages 15 to 26 during the 24 weeks of incubation. To monitor the growth until hatching, only the CLs were examined during the 3rd week to the 24th week of incubation (Table 3). In the logistic regression analyses, significant relationships between the incubation time and the CL of *M. macrocephala* were found in all three incubating temperatures with R^2 values of 0.91 (26 °C and 32 °C) and 0.87 (29 °C; Figure 5).

Based on the logistic regression analysis between the incubation time *vs.* the developmental stage (Figure 4) and between the incubation time *vs.* the CL (Figure 5), the slopes of the trend lines were compared among incubating temperatures by ANOVA followed by Tukey's multiple comparisons. The results showed that a significant difference in the slopes was found in all pair-wise comparisons ($P < 0.05$, Table 4), indicating the influence of temperature on the somatic development of the snail-eating turtle.

3.3 Effect of temperature on developmental abnormalities Upon dissection, eggs and embryos from each incubating temperature were classified into the three categories of 1) non-fertile eggs, 2) normal embryos, and 3) deformed embryos (Table 5). The proportions

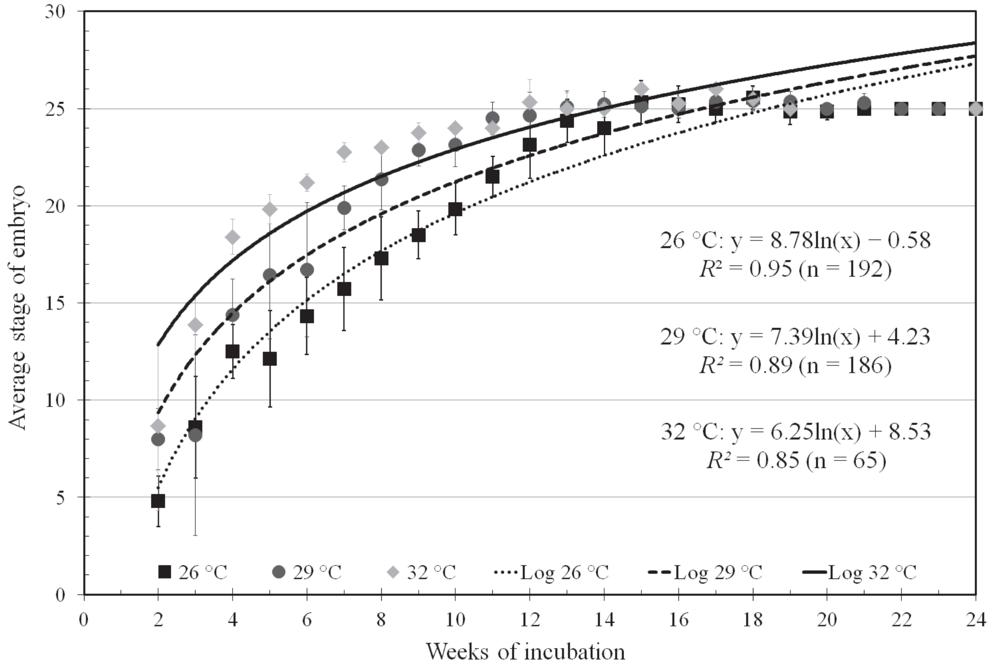

Figure 4 Effect of the incubation temperature on the growth pattern (based on embryonic stage) of *M. macrocephala* embryos. Relationships between the incubation time and average embryonic developmental stage were analyzed by logistic regression analysis, with the best fit line, equation and correlation coefficient (R^2) shown. Afterwards, the slopes of the trend lines were compared among temperatures by ANOVA followed by Tukey's multiple comparisons.

Table 1 Incubation periods for *M. macrocephala* hatching at three incubating temperatures

Temperature	Range (number of measurements) (d)	Average (mean ± SEM) (d)
Low temperature (26 °C)	89–134 (n = 40)	115 ± 11.3
Pivotal temperature (29 °C)	78–150 (n = 26)	115 ± 20.3
High temperature (32 °C)	85–131 (n = 9)	109 ± 17.8

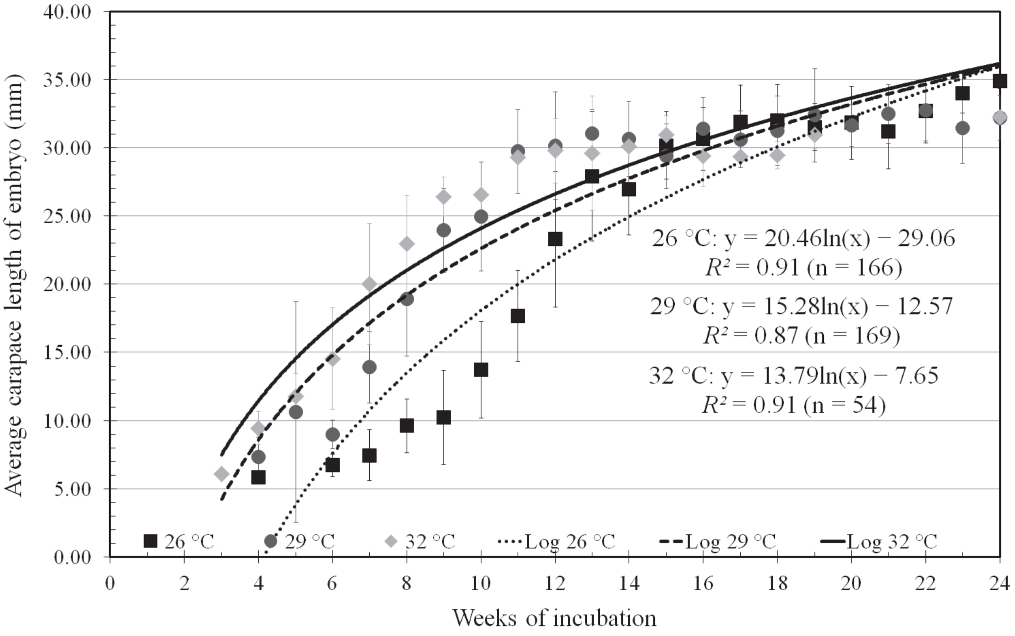

Figure 5 Effect of the incubation temperature on the growth pattern (based on carapace length) of *M. macrocephala* embryos. Relationships between incubation periods and carapace length were analyzed by logistic regression analysis, with the best fit line, equation and correlation coefficient (R^2) shown. Afterwards, the slopes of the trend lines were compared among temperatures by ANOVA followed by Tukey's multiple comparisons.

of non-fertile eggs at the three incubation temperatures were relatively similar (7.59% at 26 °C, 4.27% at 29 °C and 10.50% at 32 °C) and not statistically significantly different, as expected since fertilization, syngamy and the early cell divisions and differentiation had already occurred (or not) at ambient temperature prior to egg collection and incubation at the selected controlled temperatures. However, incubation at 32 °C resulted in a significantly higher (over 3.3-fold) proportion of abnormal embryos (72.7%) compared to that at 26 °C (19.0%) or 29 °C (21.5%). The proportions of deformed embryos at the three different incubation temperatures were also markedly different, being 0.84% at 26 °C, 2.53% at 29 °C and 30.3% at 32 °C. It is interesting to note that the elevated level of developmental mortalities observed when incubated at 32 °C were mostly of a deformed body, and so this may be a sensitive marker for thermal stress during development, although further characterization is required for verification. The 3 × 3 contingency table analysis, based on the data in Table 5, showed a significant association between the incubating temperature and the developmental normality of the embryos ($\chi^2 = 202.38$, $df = 4$, $P < 0.05$).

4. Discussion

4.1 Effect of temperature on the incubation period In this study, the time required for *M. macrocephala* eggs to hatch after incubation at 26–32 °C ranged from 78 to 150 d, which is longer than that of other freshwater turtles found in tropical regions, such as *Trachemys scripta* (65–75 d) and *Batagur baska* (70–112 d) (Ernst and Barbour, 1989). However, no significant effect of the incubating temperature on the average incubation period was found in this study.

4.2 Effect of temperature on the somatic development Growth and development of *M. macrocephala* embryos were monitored during the 24 week incubation period (egg-laying to hatching). Similar to a previous study (Vinegar, 1973), it was found that the development (based on embryonic stage) and growth (based on CL) of *M. macrocephala* embryos showed a significant logistic relationship with the incubation time. For reptilian eggs, incubation at different temperatures generally results in temperature-induced differences in the growth pattern and hatching morphology (Hutton, 1987; Packard *et al.*, 1988). For *M. macrocephala* in this study, logistic regression and slope comparison analyses supported that embryos derived from different incubating temperatures had significantly different growth patterns from each other. Incubation at a higher temperature (32 °C) resulted in relatively faster growth and development of embryos compared to that at the lower temperatures (26 °C and 29 °C). This supports previous observations that temperature is an important environment factor that affects the

Table 2 Embryonic stages[1] of *M. macrocephala* embryos after incubation at three different temperatures for 24 weeks.

Incubation time (weeks)	Embryonic stage at each incubation temperature[2]		
	26 °C	29 °C	32 °C
2	5 ± 1.30 (n = 5)	8 ± 1.58 (n = 5)	9 ± 4.32 (n = 6)
3	9 ± 2.61 (n = 5)	10 ± 5.17 (n = 5)	14 ± 1.55 (n = 8)
4	13 ± 1.38 (n = 6)	14 ± 1.82 (n = 5)	18 ± 0.89 (n = 5)
5	12 ± 2.48 (n = 7)	16 ± 3.26 (n = 7)	20 ± 0.75 (n = 6)
6	14 ± 1.97 (n = 6)	17 ± 3.45 (n = 7)	21 ± 0.45 (n = 5)
7	16 ± 2.14 (n = 7)	20 ± 1.13 (n = 8)	23 ± 0.50 (n = 4)
8	17 ± 2.14 (n = 7)	21 ± 1.60 (n = 8)	23 ± 0.00 (n = 3)
9	19 ± 1.22 (n = 6)	23 ± 0.83 (n = 8)	24 ± 0.50 (n = 4)
10	20 ± 1.33 (n = 6)	23 ± 1.13 (n = 8)	24 (n = 1)
11	22 ± 1.05 (n = 6)	25 ± 0.84 (n = 6)	24 (n = 1)
12	23 ± 1.73 (n = 8)	25 ± 1.21 (n = 11)	25 ± 1.15 (n = 3)
13	24 ± 1.12 (n = 11)	25 ± 0.83 (n = 11)	25 ± 0.82 (n = 4)
14	24 ± 1.41 (n = 11)	25 ± 0.67 (n = 9)	25 (n = 1)
15	25 ± 1.11 (n = 21)	25 ± 0.33 (n = 9)	26 ± 0.00 (n = 2)
16	25 ± 0.94 (n = 18)	25 ± 0.50 (n = 9)	25 ± 0.71 (n = 4)
17	25 ± 0.67 (n = 10)	25 ± 0.50 (n = 14)	26 (n = 1)
18	26 ± 0.60 (n = 21)	25 ± 0.51 (n = 13)	26 ± 0.58 (n = 4)
19	25 ± 0.69 (n = 7)	25 ± 0.50 (n = 11)	25 ± 0.00 (n = 2)
20	25 ± 0.41 (n = 6)	25 ± 0.00 (n = 7)	N/A[3]
21	25 ± 0.00 (n = 5)	25 ± 0.49 (n = 7)	N/A[3]
22	25 ± 0.00 (n = 6)	25 ± 0.00 (n = 7)	N/A[3]
23	25 ± 0.00 (n = 4)	25 ± 0.00 (n = 8)	N/A[3]
24	25 ± 0.00 (n = 3)	25 ± 0.00 (n = 3)	25 (n = 1)

[1] Data for deformed embryos and non-fertilized eggs (see Table 5) are excluded.
[2] Data are shown as the mean ± SEM (number of samples derived from).
[3] N/A: not available, since the embryos found in these weeks were deformed.

Table 3 Carapace length of *M. macrocephala* embryos after incubation at three different temperatures for up to 24 weeks.

Incubation time (weeks)	Carapace length (mm) at the given incubation temperature[1,2]		
	26 °C	29 °C	32 °C
3	N/A[3]	N/A[3]	6.11 ± 0.36 (n = 3)
4	5.85 (n = 1)	7.35 ± 1.41 (n = 2)	9.46 ± 1.24 (n = 5)
5	N/A[3]	10.64±10.31 (n = 5)	11.77 ± 1.66 (n = 6)
6	6.72 ± 0.81 (n = 4)	8.98 ± 1.06 (n = 6)	14.54 ± 3.72 (n = 5)
7	7.45 ± 1.86 (n = 6)	13.91 ± 2.63 (n = 8)	20.03 ± 4.44 (n = 4)
8	9.62 ± 1.98 (n = 6)	18.89 ± 4.15 (n = 8)	22.95 ± 3.57 (n = 3)
9	10.23 ± 3.45 (n = 6)	23.94 ± 3.07 (n = 8)	26.41 ± 1.42 (n = 4)
10	13.75 ± 3.54 (n = 6)	24.95 ± 4.00 (n = 8)	26.57 (n = 1)
11	17.68 ± 3.35 (n = 6)	29.74 ± 3.07 (n = 6)	29.29 (n = 1)
12	23.30 ± 4.97 (n = 8)	30.14 ± 3.94 (n = 11)	29.78 ± 2.37 (n = 3)
13	27.91 ± 4.73 (n = 11)	31.06 ± 1.71 (n = 11)	29.60 ± 4.19 (n = 4)
14	26.96 ± 3.34 (n = 11)	30.66 ± 2.74 (n = 9)	30.12 (n = 1)
15	30.15 ± 2.47 (n = 21)	29.38 ± 2.38 (n = 9)	30.93 ± 1.43 (n = 2)
16	30.64 ± 2.28 (n = 18)	31.39 ± 2.31 (n = 9)	29.42 ± 2.25 (n = 4)
17	31.91 ± 2.66 (n = 10)	30.62 ± 2.09 (n = 14)	29.34 (n = 1)
18	32.02 ± 2.64 (n = 21)	31.25 ± 2.55 (n = 13)	29.45 ± 1.00 (n = 4)
19	31.52 ± 1.72 (n = 7)	32.37 ± 3.42 (n = 11)	30.95 ± 0.98 (n = 2)
20	31.83 ± 2.65 (n = 6)	31.63 ± 1.52 (n = 7)	N/A[4]
21	31.19 ± 2.75 (n = 5)	32.48 ± 2.16 (n = 7)	N/A[4]
22	32.68 ± 2.31 (n = 6)	32.74 ± 2.26 (n = 7)	N/A[4]
23	34.02 ± 1.46 (n = 4)	31.47 ± 2.62 (n = 8)	N/A[4]
24	34.87 ± 0.53 (n = 3)	32.20 ± 1.64 (n = 3)	32.23 (n = 1)

[1] Data for deformed embryos and non-fertilized eggs (see Table 5) are excluded.
[2] Data are shown as the mean ± SEM (number of samples derived from).
[3] N/A: not available, since the embryos found in these weeks were at developmental stages 3–14 and could not be measured for carapace length.
[4] N/A: not available, since the embryos found in these weeks were deformed.

Table 4 Comparisons of the temperature-related difference in slopes (ANOVA and Tukey's multiple comparisons) of the logistic regression analysis between the incubation time and development of *M. macrocephala* based on the embryonic stage and carapace length.

Slope comparison	n	*F*	*P*	q	*P*
Comparison on slope of logistic regression between incubation time and embryonic stage					
26 °C vs. 29 °C	374	43.21	< 0.05	8.11	< 0.05
26 °C vs. 32 °C	253	43.21	< 0.05	27.25	< 0.05
29 °C vs. 32 °C	247	43.21	< 0.05	9.76	< 0.05
Comparison on slope of logistic regression between incubation time and carapace length					
26 °C vs. 29 °C	335	4.08	< 0.05	5.79	< 0.05
26 °C vs. 32 °C	220	4.08	< 0.05	2.62	< 0.05
29 °C vs. 32 °C	223	4.08	< 0.05	3.57	< 0.05

Table 5 Incidence of developmental abnormalities of *M. macrocephala* embryos incubated at three different temperatures.

State of eggs/embryos	Temperature			Total
	26 °C	29 °C	32 °C	
Total number of eggs	237	237	238	712
Non-fertile eggs	18	10	25	53
Normal embryos	192	186	65	443
Deformed embryos	2	6	72	80
Minor deformities (Type 1)				
Narrow body	1	1	2	4
Moderate deformities (Type 2)				
Scoliosis	1	0	1	2
Missing eye	0	1	4	5
Flat skull, misshapen carapace	0	2	5	7
Deformed body	0	2	55	57
Lethal deformities (Type 3)				
Dwarf	0	0	5	5

developmental rate of turtle embryos (Valenzuela, 2001; Zhu *et al.*, 2006).

However, incubation at the high temperature (32 °C) was harmful to the development of the turtle embryos since a significantly (over 3.3-fold) higher proportion of deformities were found. Interestingly, these were mainly of a deformed body (Type 2 deformities) phenotype, suggesting that this may be a developmentally sensitive stage to elevated temperature. Obviously, reptilian eggs cannot be incubated at extremely high or low temperatures for extended periods of time, since it can increase embryonic deformities (Du and Ji, 2003; Sinervo and Adolph, 1989). In turtles, the thermal condition during incubation is known to influence the incubation time, metabolism, sex, embryo mortality, hatching success and embryo size at hatching (Standing *et al.*, 2000). This study provides further evidence that increases in the incubating temperature could result in a higher incidence of developmental abnormalities.

5. Conclusion

Incubation temperature is an important variable affecting the development of freshwater turtles. In *M. macrocephala*, the incubation temperature was seen to affect the somatic development of the embryos. At a high temperature (32 °C) a significantly more rapid growth and development rate was found than at 26 °C and 29 °C, but with a significantly (over 3.3-fold) higher incidence of deformities.

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Review Number 1323005).

References

Bell B., Spotila J. R., Congdon J. 2006. High incidence of deformity in aquatic turtles in the John Heinz National Wildlife Refuge. Environ Pollut, 142: 457–465

Booth D. T. 2006. Influence of incubation temperature on hatchling phenotype in reptiles. Physiol Biochem Zool, 79: 274–281

Brophy T. R. 2004. Geographic variation and systematics in the southeast Asia turtle of the genus *Malayemys* (Testudines: Bataguridae). Hamadryad, 29: 63–79

Bull J. J., Wibbels T., Crews D. 1990. Sex-determining potencies vary among female incubation temperature in a turtle. J Exp Zool, 256: 339–341

Davy C. D., Murphy W. M. 2009. Explaining patterns of deformity in freshwater turtles using MacCulloch's hypothesis. Can J Zool, 87: 443–439

Deeming D. C., Ferguson M. W. J. 1991. Egg incubation: Its effects on embryonic development in bird and reptile. New York: Cambridge University Press

Du W. G., Ji X. 2003. The effects of incubation thermal environments on size, locomotor performance and early growth of hatchling soft-shelled turtle, *Pelodiscus sinensis*. J Therm Biol, 28: 279–286

Ernst C. H., Barbour R. W. 1989. Turtle of the world. Washington, D.C.: Smithsonian Institution Press.

Ewert M. A. 1979. The embryo and its egg: Development and natural history. In Harless M., Morlock H. (Eds.), Turtles: Perspectives and Research. New York: Wiley, 333–413

Ewert M. A. 1985. Embryology of turtle. In Gans C., Billet F. S., Maderson P. F. A. (Eds.), Biology of the Reptilia, Vol. 14, Development A. New York: Wiley, 75–267.

Georges A., Beggs K., Young J. E., Doody J. S. 2005. Modeling development of reptile embryos under fluctuating temperature regimes. Physiol Biochem Zool, 78: 18–30

Grant M., Ashmore F., Janzen J. 2003. Phenotypic variation in smooth softshell turtle (*Apalone mutica*) from egg incubation in constant versus fluctuating temperatures. Oecologia, 134: 182–188

Hutton J. M. 1987. Incubation temperatures, sex ratios and sex determination in population of Nile crocodiles (*Crocodylus niloticus*). J Zool Lon, 211: 143–155

Intergovernmental Panel on Climate Change (IPCC). 2007. Climate change 2007: Synthesis report. Contribution of working group I, II and III to the fourth assessment report of the Intergovernmental Panel on Climate Change. IPCC, Geneva.

Keithmaleesatti S. 2008. An assessment of the association of organochlorine pesticide contamination and reproductive effects on the snail-eating turtle (*Malayemys macrocephala*) in the lower Chao Phraya River basin, Thailand. Ph.D. Dissertation. Chulalongkorn University. 143 pp.

MacCulloch R. 1981. Variation in the shell of *Chrysemys picta belli* from southern Saskatchewan. J Herpetol, 15: 181–185

Nutphand W. 1979. The turtles of Thailand. Bangkok: Siamfarm Zoological Garden

Packard G. C., Packard M. J., Miller K., Boardman T. J. 1987. Influence of moisture, temperature, and substrate on snapping turtle eggs and embryos. Ecology, 68: 983–993

Packard G. C., Packard M. J., Miller K., Boardman T. J. 1988. Effect of temperature and moisture during incubation on the composition of hatching snapping turtles (*Chelydra serpentina*). J Comp Physiol, 158: 117–125

Parrott A., Logan J. D. 2010. Effects of temperature variation on TSD in turtle *Chrysemys picta* populations. Ecol Model, 221: 378–1393

Pavaliko P. 1986. Shell and scute anomalies in some Midwestern turtles. Bull Chicago Herpetol Soc, 21: 36–38

Pieau C., Dorizzi M. 1981. Determination of temperature sensitive stage for sexual differentiation of the gonads in embryos of the turtle *Emys orbicularis*. J Morphol, 170: 373–382

Pina C., Larriera A. 2002. *Caiman latirostris* growth: The effect of a management technique on the supplied temperature. Aquaculture, 211: 387–392

Sinervo B., Adolph S. C. 1989. Thermal sensitivity of growth rate in hatchling *Sceloporus* lizard: Environmental, behavioral and genetic aspects. Oecologia, 78: 411–419

Srinarumol N. 1995. Population biology of the Malayan snail-eating turtle *Malayemys subtrijuga*. M.Sc. Thesis. Chulalongkorn University. 126 pp

Standing K. L., Herman T. B., Morrison I. P. 2000. Developmental abnormalities in a northeastern population of Blanding's turtle, *Emydoidea blandingii*. Chelonian Cons Biol, 3: 661–664

Thirakhupt K., Van Dijk P. P. 1994. Species diversity and conservation of turtles of western Thailand. Nat Hist Bull Siam Soc, 42: 207–259

Tokita M., Kuratani S. 2001. Normal embryonic stage of the Chinese softshelled turtle *Pelodiscus sinensis* (Trionychidae). Zool Sci, 18: 705–715

Valenzuela N. 2001. Constant, shift and natural temperature effects on sex determination in *Podocnemix expansa* turtles. Ecology, 82: 3010–3024

Vinegar A. 1973. The effect of temperature on the growth and development of embryo of the Indian python, *Python molurus* (Reptilia, Serpentes: Boidae). Copeia, 1973: 171–173

Walther G. R., Post E., Convey P., Menzel A., Parmesank C., Beebee T. J. C., Fromentin J. M., Guldbergi O. H., Bairlein F. 2002. Ecological responses to recent climate change. Nature, 416: 389–395

Willingham E. 2005. Different incubation temperatures result in differences in mass in female red-eared slider turtle hatchlings. J Therm Biol, 30: 61–64

Yntema C. L. 1968. A series of stage in the embryonic development of *Chelydra serpentina.* J Morphol, 125: 219–252

Zar H. J. 1998. Biostatistical analysis, 4th Ed. New Jersey: Prentice Hall International

Zhu X. P., Wei C. Q., Zhao W. H., Du H. J., Chen Y. L., Gui J. F. 2006. Effect of incubation temperatures on embryonic development in the Asian yellow pond turtle. Aquaculture, 259: 243–248

Zug G. R., Vitt L. J., Caldwell J. P. 2001. Herpetology: An introductory biology of amphibians and reptiles, 2nd Ed. California: Academic Press